REMARKS

Reconsideration of this Application and entry of the foregoing amendments are requested. Claims 4 and 12 have been amended in view of the Office Action and to better define what the Applicants consider their invention, as fully supported by an enabling disclosure. New claim 21 has been added. Additional support for the amendments to claim 4, 12 and 21 can be found, for example, in original claims 1, 12 and 4, respectively.

The Examiner is referred back to our response of February 25, 2002, which it is respectfully submitted addressed and overcame most if not all rejections of Examiner Sisson.

Applicants are concerned by the lack of advancement in this case in view of the lack of factual support, objective evidence or lack of comprehension of the invention by the Examiner. The re-iteration of "several man year's", "with little, if any, reasonable expectation of success", "The guidance provided by the specification is limited to specific genes of a single species of bacteria" and then the "selective condition is not readily apparent". The misunderstanding of kanamycin as a "selective condition" as in the meaning of claim 4 are but limited examples of the above-mentioned lacks of progress and comprehension of the Examiner. Applicants will try once again to provide further clarification to advance the prosecution of this case as follows.

REJECTIONS UNDER 35 U.S.C. § 112 FIRST PARAGRAPH

The Examiner continues to maintain that claims 4 to 12 contain matter not sufficiently described in the application to enable one skilled in the art to make and/or use the invention. In particular, under the heading "The Presence or Absence of Working Examples", the Examiner notes the following: "Upon review of Example 1, it appears that the "selective condition" employed was the inclusion of kanamycin in the culture media. However, the cells demonstrated resistance to kanamycin even after having undergone mutagenesis. Accordingly, the "selective condition" did not select for one group of cells over that of another." It appears from this extract of the Examiner's comments that it is not clear to him what the test described in Example 1 is meant to show and what the role of kanamycin is in this test. The following statements seek to clarify what the EGT assay is and what conclusions may be inferred from its results:

1) The premise of one of the embodiments of the present invention is that a clone having one of its essential genes disrupted will not grow. In the same manner, a clone having an insertion in a non-essential gene will grow.

2) The selective condition as encompassed by one method of the present invention is applied to determine which genes are essential under that selective condition. Under such selective condition, if a clone having an insertion in a specific gene does not grow at all or grows to a lesser extent than that same clone under non-selective condition, it shows that this gene is essential under that selective condition.

3) Example 1 seeks to demonstrate that the absence of the extension product containing the insertion sequence (i.e. and therefore the absence of growth of clones containing an insertion product in the targeted *ftsZ* gene) shows that this gene is essential to the growth of the clone since the clone having that gene disrupted was not able to grow. Similarly, the presence of an extension product containing the insertion sequence (i.e. and therefore the growth of the clone containing an insertion in the *ampC* gene) indicates that this gene was not essential since the clone was able to grow notwithstanding the disruption in this gene.

4) In Example 1, the essential gene ftsZ was shown to be essential using as mutagen the transposable element miniTn5Km ("using standard methods" p. 26, lines 3-6). As very well known to a person of ordinary skill to which the present invention pertains, insertion of the miniTn5Km insertion element into a genome disrupts the region into which it is inserted and confers resistance to Km (Kanamycin) to the cell which harbors this insertion element. Km resistance is thus used initially to select the pool of mutagenized clones that has been calculated to achieve saturation mutagenesis.

For certainty, Example 2 uses a different insertion element for mutagenesing the targeted region: miniTn5tet, which confers tetracycline resistance. The Examiner is referred more particularly to page 27, lines 30-31.

The Examiner further indicates in item 4 that the specification does not provide support to enable one skilled in the art to practice the full scope of the invention. He further states that no evidence is provided by the Applicants refuting the Examiner's assertion. He finally indicates that the specification does not provide guidance as to how the standard techniques should be modified for other organisms. These techniques are standard techniques that have been used for decades. Some of which like mutagenesis, selective condition and the like were instrumental in laying the foundation of biotechnology 50 years ago. See for instance enclosed p. 15.1 to 15.13 of Sambrook *et al*.1989 for the mutagenesis by insertion; enclosed p. 1.74-1.105 for host cell transformation, enclosed p. 1.21 to 1.24 of Sambrook for transformant cells growth; enclosed p.

14.1 to 14.33 for amplification of DNA sequences; enclosed chapter 6, p. 6.1-6.27 of Sambrook *et al*.1989 for gel electrophoresis of DNA.

The Examiner maintains in item 5 that he considers that the examples with *Pseudomonas aeruginosa* used to exemplify the claimed invention are not sufficient to enable the full scope of the invention, and in particular to organisms containing intronic sequences. The Applicants respectfully disagree. The scope of the method of the present invention should not be limited to prokaryotes since the technique for mutagenizing prokaryotes described in the specification at pages 21, line 21 to p. 22, line 8; at p. 23, line 19 to p. 24, line 3; and particularly for *Pseudomonas* at p. 27, line 12 to p. 298, line 29, may be adapted by following the guidelines specified in the specification at p. 21, lines 5 to 11 as follows "the method of the present invention relates particularly to genomes of organisms which do not contain or contain few introns, the present invention could be adapted by a person of ordinary skill for intron-containing genomes. Briefly, the level of mutagenesis would have to be increased in order to enable saturation to occur"; The cited references Sambrook et al., 1989 and Directed Mutagenesis: A Practical Approach, 1991, respectively, teach how mutagenesis of organisms having intronic sequences may be performed.

The Examiner further indicates in item 5 that "the specification has not set forth in sufficient detail how the claimed invention is to be practiced with any and all source of genetic material." The Applicants respectfully disagree. While it is true that the present application comprises examples wherein the essentiality or non-essentiality of known genes were determined or confirmed, these examples were used specifically to show that the methods of the present invention are indeed accurately characterized genes as being essential or not: they correctly determine that gene *asd* is essential and that genes *ampC*, *algK* and *rcf* are not. However the test was also used to determine whether genes *ftsZ*, *ddl*, *ftsQ* and *ftsA*, the essentiality or non-essentiality of which are not known. Furthermore, the specification indicates that the present method may be used to determine the essentiality or non-essentiality of genes that are not known if, as indicated at p. 19, lines 14-19 of the specification, the "DNA region for which preliminary sequence data is available sufficiently to enable the design of a first primer pair which will, under appropriate conditions, give rise to a recognizable extension product. The target region is determined and defined by the available sequence data available for the particular genome analysed". Therefore, any gene for which preliminary sequence data is known can be identified

as essential or non-essential according to the present invention. Putative open reading frames may be determined from any published genomes of organisms and may serve as target sequences for the present invention.

The Examiner indicates in item 6 that he is not convinced with the argument presented in the last response, which stated that the "claimed method has nothing to do with predicting the properties of proteins." He indicates that this is not persuasive since "as seen in claim 11, one is looking for resistance to cytotoxic agents. Clearly resistance is manifest in the protein so encoded." The Applicants respectfully submit that the EGT test of the present invention does not assess protein expression *per se* and does not need to assess it in order to determine whether genes are essential or not. As explained above, the EGT test determines whether a gene is essential or not simply by determining the absence or presence of gene sequences containing the insertion. The presence of these disrupted sequences shows survival of cells containing the same and therefore non-essentiality of the disrupted gene. Further details on how to interpret results of the EGT assay are presented at p. 25, lines 6 to 24 of the specification.

The Examiner indicates in items 7 and 8 that the level of skill required to enable one skilled in the art to fully practice the invention (i.e. identify whether <u>any</u> gene is essential or non essential) rises to undue experimentation. The Applicants believe that the arguments and support provided in relation to item 4 above fully respond to this objection.

In view of the above and foregoing, it is respectfully requested that the Examiner withdraws his rejection of claims 4 to 12 under 35 U.S.C. §112, first paragraph.

The Examiner maintains his rejection of claims 4 to 12 pursuant to 35 USC § 101. He alleges in items 10-13 that the claimed invention has no specific or credible utility. The Applicants respectfully disagree. The specification sets forth a number of utilities for specific embodiments of the method of the present invention. In particular, it sets forth in the field of the invention that the essential genes thus identified may serve as new "therapeutic targets and more specifically to therapeutic targets in bacteria". Indeed, such analysis could be, for example, the next phase in microbial genomics, particularly as it pertains to finding novel therapeutic targets in bacteria. See also p. 27, lines 7 to 11 to the same effect. The Examiner is also referred to p. 24, lines 11 to 12; and 16-18 specifying that the EGT assay can be applied to all disease causing organisms and be "utilized to dissect metabolic and genetic pathways by assessing mutagenized populations in different *in vitro* and *in vivo* conditions."

In view of the above and foregoing, it is respectfully requested that the Examiner withdraws his rejection of claims 4 to 12 under 35 U.S.C. §101.

The rejections of the previous claims are believed to have been overcome by the present remarks and the amendment of the claims. From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such an action is earnestly solicited.

Authorization is hereby given to charge deposit account no. 13-2725 for any deficiencies or overpayment in connection with this response.

If the Examiner feels that a telephone interview may be helpful in this matter, please contact Applicants' Representative at 612.336.4728.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 4 and 12 have been amended as follows: Underlines indicate insertions and ~~strikeouts~~ indicate deletions.

4. (AMENDED) A method for functional analysis of a target region in a sequence of interest of an haploid genome, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence tag is inserted into said target region such that a population of DNA molecules is obtained having at least 90% of nucleotide positions of said target region insertionally mutated; [mutagenizing said target region by insertion of a sequence tag to provide a population of DNA molecules containing a sequence tag insertion in at least 90% of nucleotide positions in said target region]

introducing said population of mutagenized DNA molecules into host cells that express said sequence of interest;

subjecting a first aliquot of said host cells to at least one selective condition and a second aliquot to a non-selective condition [to provide]thereby providing at least one selected and one non-selected aliquot;

amplifying said target region from said at least one selected and one non-selected aliquots, using a first primer hybridizing to said sequence tag and a second primer hybridizing to a known endpoint, said endpoint being characterized as an arbitrary unique sequence in said target DNA, [to provide]thereby giving rise to amplified DNA; and

resolving by gel electrophoresis said amplified DNA from said at least one selected and one non-selected aliquots into individual bands [differing]which differ by size, to identify the position of individual sequence tag insertions within said target region,

whereby differences between the presence or intensity of bands between said at least one selected and one non-selected aliquots are indicative that said sequence tag insertion causes a difference in response to said selective condition thereby functionally identifying said target

region under said at least one selected condition [employed with said at least one selected aliquot resulting in the functional analysis of said target region].

12. (AMENDED) A method according to [one of] claim[s] 4[to 11], whereby the absence of a band under said selective condition and its presence under non-selective conditions is indicative of a target region which is essential under said selective condition.

21. (NEW)A method according to claim 4, wherein said haploid genome is a bacterial genome.